Distribution of viral antigen and development of lesions after experimental infection of calves with a BVDV 2 strain of low virulence

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Abstract. To examine the virus-host interaction in subclinical bovine viral diarrhea virus (BVDV) infections, the spread of a BVDV 2 strain of low virulence to different organs and the development of lesions were investigated. Eight colostrum-deprived, clinically healthy, 2–3-month-old calves were intranasally inoculated with 10^6 tissue culture infective dose of the naturally occurring BVDV 2 strain 28508-5 of low virulence, and 2 served as controls. Two calves each were euthanized at days 3, 6, 9, and 13 postinoculation (pi). Representative tissues were processed for histology and immunohistology. Signs of overt clinical disease were absent. However, a mild temperature elevation at days 7 or 8 pi and a moderate decrease of circulating lymphocytes occurred in all inoculated calves. The BVDV antigen was detected at day 3 pi in several lymphoid tissues. At day 6 pi, BVDV antigen was found widespread in lymphoid tissues and multifocally in intestinal epithelial cells but was associated with no or subtle lesions only. At day 9 pi, much less BVDV antigen was detectable, but there was severe depletion of lymphoid tissues. At day 13 pi, BVDV antigen had been cleared from most lymphoid tissues that were at variable phases of depletion and recovery. In conclusion, the BVDV strain of low virulence spread to lymphoid tissues and intestinal epithelial cells but was rapidly eliminated. Transient depletion of lymphoid tissues was followed by recovery.

Bovine viral diarrhea virus (BVDV) is a *Pestivirus* in the family Flaviviridae.[17] Infections with BVDV are widespread in cattle herds and cause severe economical losses in North America and Europe despite intense efforts to control or eradicate them.[19] On the basis of genomic differences in the 5′NTR region of BVDV, 2 species, BVDV 1 and BVDV 2, are currently differentiated.[4,17,33] The BVDV strains can grow in epithelial cell cultures with or without inducing a cytopathic (cp) effect, thus allowing further grouping in cp and noncytopathic (ncp) biotypes.[13,32]

Infections with BVDV of both genotypes have been associated with a wide variety of clinical conditions including subclinical infection, reproductive failure, severe acute disease with or without hemorrhagic diathesis, and mucosal disease.[3] During the last decade, an increased number of field cases of severe acute BVD in all age groups of cattle has been reported.[8,10,18,30] The BVDV isolates from these outbreaks in North America predominantly belong to BVDV 2.[8] Besides the highly virulent strains of BVDV 2, there also exist strains causing subclinical infections only.[35] These strains might be even more important for the

spread of BVDV because they may circulate undetected in a herd for an extended time period and may predispose to secondary infections or disease.

Highly virulent isolates from field cases of severe acute BVD have been used to investigate the pathogenesis and pathology of acute BVD,[1,5,9,12,16,25,28,37] but there exists only 1 study using a BVDV 2 strain of low virulence.[27] It is still unclear how virus–host interactions differ between strains of high and low virulence. The aim of this study was to determine the extent to which an ncp BVDV 2 strain of low virulence replicates and alters tissues. Therefore, spread of viral antigen and development of tissue lesions were examined at sequential time points after inoculation of colostrum-deprived calves.

Material and methods

Animals. Ten 2–3-mo-old, colostrum-deprived, clinically healthy male calves of different breeds (8 Holstein Frisian, 1 Brown Swiss, and 1 Ayrshire) were used. All calves were negative for BVDV and antibodies to BVDV as determined by virus isolation from buffy coat followed by polymerase chain reaction (PCR)[34] and serum neutralization using BVDV 1 strain NY-1 and BVDV 2 strain 1373, respectively, before inoculation.

Inoculum. The ncp BVDV 2 strain 28508-5 was used. This strain was isolated from the tissues of a clinically normal, persistently infected calf. The virus used for inoculation had been passed once in calves

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followed by 2 passages in tissue culture. Preceding experimental infections had produced no signs of disease in calves.[35]

Experimental procedure. Eight calves received 5 ml containing 10^6–10^7 tissue culture infective dose of BVDV 2 strain 28508-5 intranasally. The body temperature was monitored daily. Blood samples were collected at the time of inoculation and at days 3, 6, 9, and 13 postinoculation (pi). In these samples the number of white blood cells, lymphocytes, and platelets was determined, and the buffy coat was examined for BVDV by virus isolation followed by PCR.[34] Two calves each were euthanized and necropsied at days 3, 6, 9, and 13 pi. Two calves were not inoculated with BVDV and served as controls. They were necropsied at the end of the experiment.

Histology and immunohistology. At necropsy, tissue samples were collected from 38 locations including lymphoid organs (tonsil, mandibular lymph node, retropharyngeal lymph node, subscapular lymph node, mesenteric lymph node, spleen, and thymus), digestive tract (oral mucosa, esophagus, rumen, abomasum, duodenum, jejunum, colon, Peyer's patches in jejunum and ileum, gut-associated lymphoid nodules at the ileocecal junction, in the proximal colon and at the rectum, parotid salivary gland, pancreas, and liver), respiratory tract (nasal orifice, turbinates, trachea, and lung), endocrine organs (thyroid, pituitary, pancreas, and adrenal), urogenital organs (kidney and testis), central nervous system (cerebrum, hippocampus, and cerebellum), heart, skin, interdigital skin, and bone marrow. One half of each tissue sample was fixed in 5% neutral buffered formalin, processed, embedded in paraplast, and sectioned. The other half of the tissue sample was snap-frozen in 2-methylbutane[a] at −70 C for immunohistology.

Viral antigen was detected in cryostat sections by the indirect immunoperoxidase method as described.[22] The monoclonal antibody BZ81 against E2 was used as primary antibody diluted 1:10 in phosphate buffered saline (pH 7.1) containing 1% polyoxyethylene sorbitan monolaurate[b,c]. Sections were incubated in a humid chamber for 1 hr at room temperature.

The paraplast sections were stained with hematoxylin and eosin (HE). Sites where viral antigen had been detected in frozen sections (all lymphoid organs, gut-associated lymphoid nodules in small and large intestine) and the bone marrow were examined for changes in cellular proliferation. The monoclonal mouse anti-human Ki-67 antigen (clone MIB-1) antibody[d] was used to detect the nuclear proliferation-associated antigen Ki-67 in paraplast sections by the indirect immunoperoxidase method as described.[24] Changes in the number of Ki-67-positive cells were used to confirm alterations seen in HE-stained sections and were not scored.

Results

Clinical signs. No changes in behavior and appetite and no signs of disease were seen in any of the inoculated or control animals. An increase of body temperature to above 40 C occurred at day 7 and 8 pi (calf No. 8) or at day 8 pi only (calf Nos. 7, 9, and 10). Temperature had returned to normal values by day 9 pi (Fig. 1).

Circulating lymphocytes were decreased, resulting in an average reduction of 33% (18–52% range) at day 3 pi and 38% (24–53% range) at day 6 pi. At days 9 and 13, lymphocyte numbers were on average 28% and 20% below the baseline values; however, the numbers were within the range of variation seen in the controls.

Platelet numbers were within the clinically normal range in all calves throughout the experiment. In 6 of the infected animals and in the controls, only mild day-to-day fluctuations were observed. In 2 calves, there was a decrease of platelet numbers to 52% at day 6 (calf No. 6) and to 48% at day 9 (calf No. 8).

The BVDV was isolated from buffy coat samples at day 3 pi from 1 (calf No. 10) of 8 infected animals. At day 6 pi, the majority of the remaining calves (4 of 6, calf Nos. 6, 7, 9, and 10) was viremic. The BVDV was not isolated from any animal at days 9 or 13 pi or at any time point from the noninfected controls (calf Nos. 1 and 2) or the infected calves (calf Nos. 3, 4, 5, and 8).

Macroscopic findings. The control animals, both calves at day 3 pi and 1 of the calves at day 6 pi (calf No. 5), had very large lymph nodes and tonsils as well as prominent gut-associated lymphoid tissue in the small and large intestine. In the other calf at day 6 pi (calf No. 6), as well as both calves at day 9 pi and both calves at day 13 pi, the lymphoid tissues were slightly smaller or thinner. Multiple 2–5 mm large, white nodules in the renal cortex indicative of non-suppurative interstitial nephritis were present in 4 calves (calf Nos. 3, 4, 6, and 7), and an abscess in a mesenteric lymph node in 4 (calf No. 1) was present.

Distribution of viral antigen. The BVDV antigen was not detected in tissues or white blood cells of either control animal. In the inoculated calves, BVDV antigen was present only in lymphoid tissues and intestinal mucosa (Table 1). The BVDV antigen was never detected in the upper digestive tract, including forestomachs and abomasum, in the upper and lower respiratory tract, in organs of the endocrine system, in the central nervous system, kidney, liver, heart, testis, skin, interdigital skin, and bone marrow of any of the infected animals.
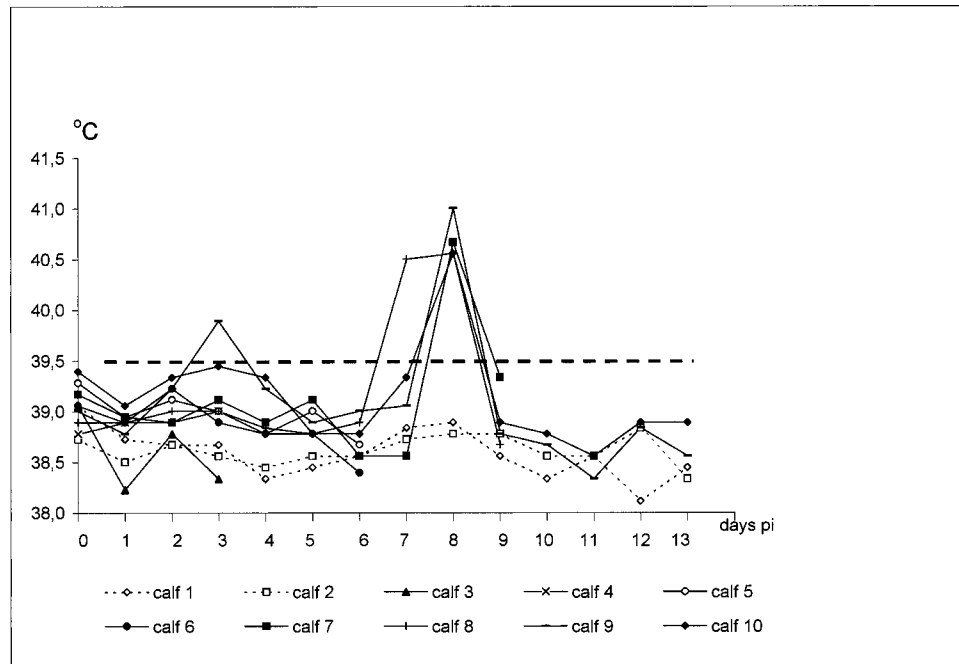
Figure 1. Body temperature after inoculation with BVDV 2 strain 28508-5.

In both calves necropsied at day 3 pi (calf Nos. 3 and 4), BVDV antigen was present in epithelium, lymphocytes, and cells with dendritic morphology in the subepithelial and interfollicular areas of the tonsils (Fig. 2) as well as in lymphocytes and cells with dendritic morphology in the subcapsular sinuses and paracortex of the retropharyngeal and mesenteric lymph nodes (Table 1). The BVDV antigen was also detected in lymphocytes and follicular dendritic cells of a few lymphoid follicles in the tonsil and lymph nodes of calf No. 4. In calf No. 3, BVDV antigen was also found in the mandibular and subscapular lymph node. Viral antigen was present in the interfollicular areas and domes of jejunal Peyer's patches and lymphoid nodules at the ileocecal junction and in the proximal colon of both calves (calf Nos. 3 and 4), and in the

Table 1. Organ and tissue distribution of antigen after inoculation with BVDV 2 strain 28508-5.

|	Control		3 dpi*		6 dpi		9 dpi		13 dpi	
Animal number	1	2	3	4	5	6	7	8	9	10
Lymphoid tissues										
Tonsil	−	−	+	+	+	+	+	−	+	−
Mandibular lymph node	−	−	+	−	+	+	−	−	−	−
Retropharyngeal lymph node	−	−	+	+	+	+	+	−	−	−
Superficial cervical lymph node	−	−	+	−	+	+	−	−	−	−
Mesenteric lymph node	−	−	+	+	+	+	+	−	−	−
Spleen	−	−	−	−	+	+	−	−	−	−
Thymus	−	−	−	−	+	+	+	+	−	+
Bone marrow	−	−	−	−	−	−	−	−	−	−
Intestines										
Duodenum	−	−	−	−	−	+	−	−	−	−
Jejunum	−	−	−	−	−	−	−	−	−	−
Jejunum with Peyer's patch	−	−	+	+	+	+†	+	+	−	−
Ileum with Peyer's patch	−	−	−	−	+†	+†	+	+	−	−
Ileocecal junction	−	−	+	+	+	+	+	−	−	−
Colon with lymphoid nodule	−	−	+	+	+	+†	+	−	−	−
Midcolon	−	−	−	−	−	+†	−	−	−	−
Rectum with lymphoid nodule	−	−	−	+	+†	+†	+	−	−	−

* dpi = days postinoculation.
† Positive epithelial cells in addition to positive cells in the lamina propria and lymphoid tissue.
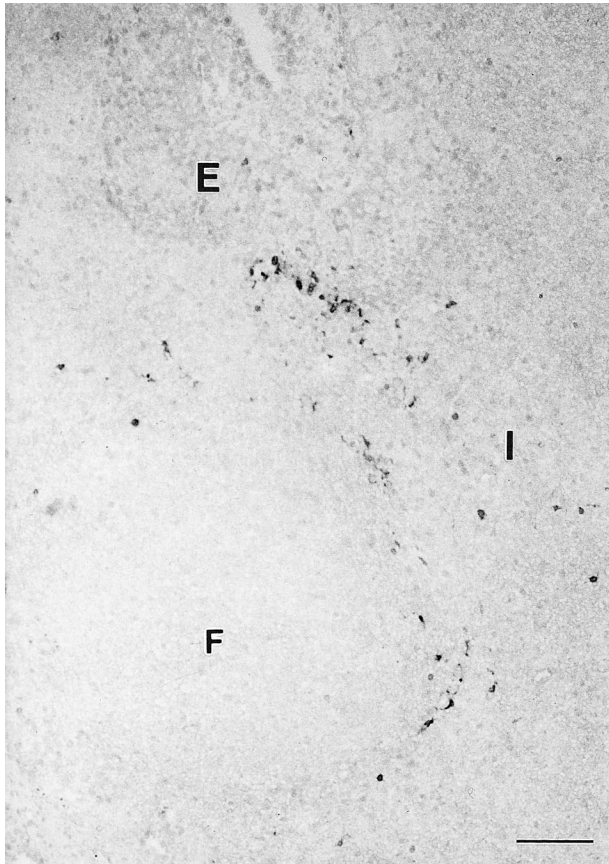
Figure 2. Distribution of BVDV antigen in the tonsil at day 3 pi (calf No. 3). Viral antigen is present subepithelially, in the interfollicular area (I) and in the periphery of the lymphoid follicle (F). There is no viral antigen in this area of tonsillar epithelium (E). Bar = 100 μm.
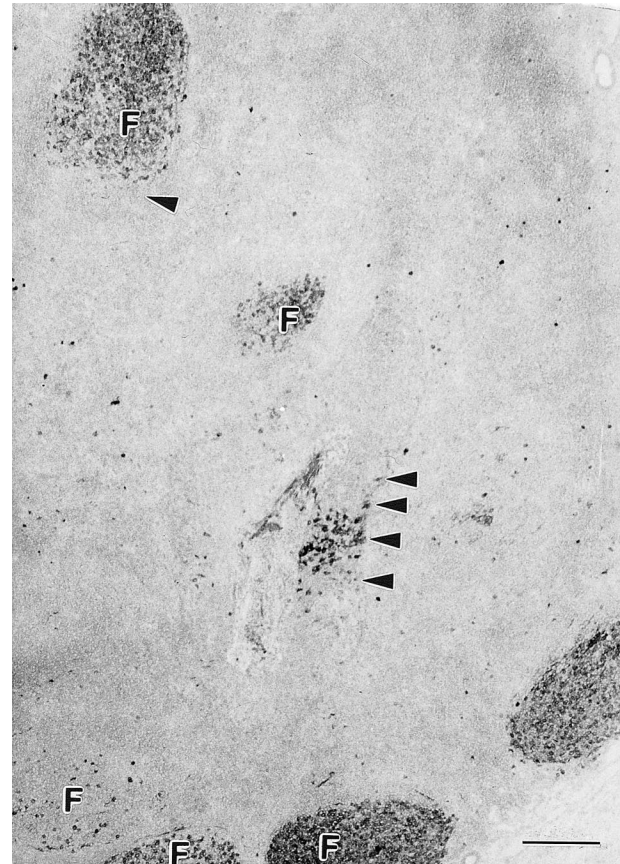
Figure 3. Distribution of BVDV antigen in the tonsil at day 6 pi (calf No. 6). Viral antigen is predominantly present in lymphoid follicles (F) and in the tonsillar epithelium (arrowheads). The number of cells containing viral antigen varies from very few to almost all cells between individual lymphoid follicles. Bar = 200 μm.

lymphoid nodules in the rectum of calf No. 4. Viral antigen was distributed in a multifocal pattern in tonsils and retropharyngeal lymph nodes, whereas 1–3 foci were found in the other lymph nodes and in the gut-associated lymphoid tissues only.

At day 6 pi, the widest distribution and the highest numbers of cells containing BVDV antigen were seen (Table 1). In both calves (calf Nos. 5 and 6), BVDV antigen was present in the tonsils, all the lymph nodes collected, spleen, thymus, jejunal and ileal Peyer's patches, as well as in gut-associated lymphoid tissue at the ileocecal junction, proximal colon, and rectum. In addition, cells containing viral antigen were found in the duodenum and midcolon of calf No. 6. The following distribution of viral antigen was seen in these organs. In the tonsils, most lobules contained BVDV antigen predominantly in lymphoid follicles, in multiple epithelial cells, especially in areas with reticulate epithelium, and less frequently in lymphocytes and cells with dendritic morphology in the subepithelial and interfollicular areas (Fig. 3). In infected lymphoid follicles, BVDV antigen was detected in both lympho-

cytes and follicular dendritic cells; the number of positive cells varied from very few to almost all cells within the follicles. In most lymphoid follicles of the retropharyngeal lymph nodes, many of the mandibular and mesenteric lymph nodes, and few of the subscapular lymph nodes, BVDV antigen was present in lymphocytes and follicular dendritic cells (Fig. 4). As described for the tonsil, there were marked differences in the frequency of BVDV-positive cells between infected lymphoid follicles in the lymph nodes. A few individual cells containing BVDV antigen were also seen in the subcapsular, trabecular, and medullary sinuses as well as throughout the paracortex of the lymph nodes (Fig. 4). In the spleen, BVDV antigen was detected in lymphocytes and follicular dendritic cells of 1 lymphoid follicle in calf No. 5 and most lymphoid follicles in calf No. 6. The BVDV antigen was distributed multifocally in lymphocytes and cells with dendritic morphology in cortex and medulla of a few thymic lobules (Fig. 5).

Lymphoid follicles containing BVDV antigen were seen multifocally in jejunal and ileal Peyer's patches,

Figure 4. Distribution of BVDV antigen in the cortex of a mandibular lymph node at day 6 pi (calf No. 5). Viral antigen is predominantly present in lymphocytes (arrow, example) and follicular dendritic cells (arrowhead, example) in the lymphoid follicle. A few positive cells are present in the subcapsular sinus (open arrows) and in the paracortex (long arrow). Bar = 50 μm.
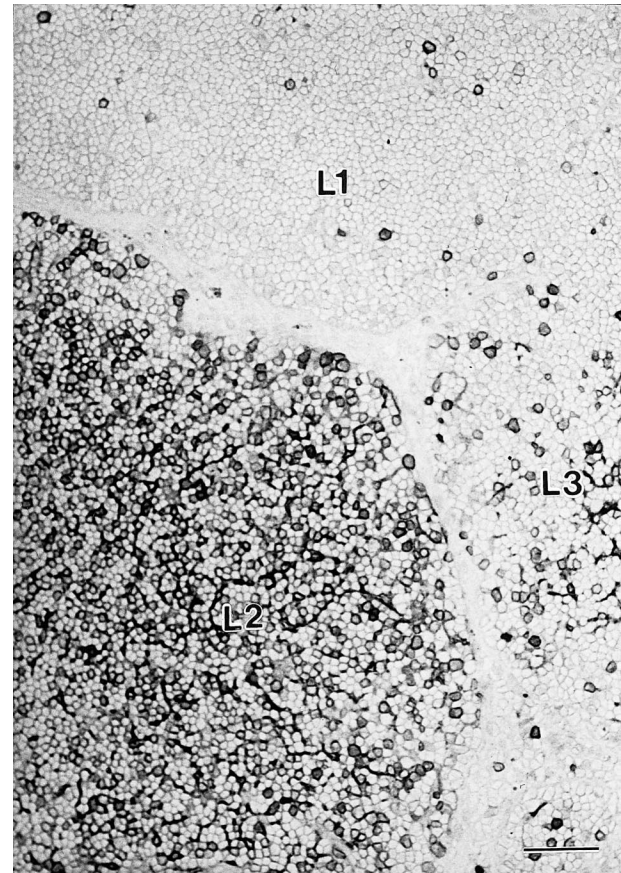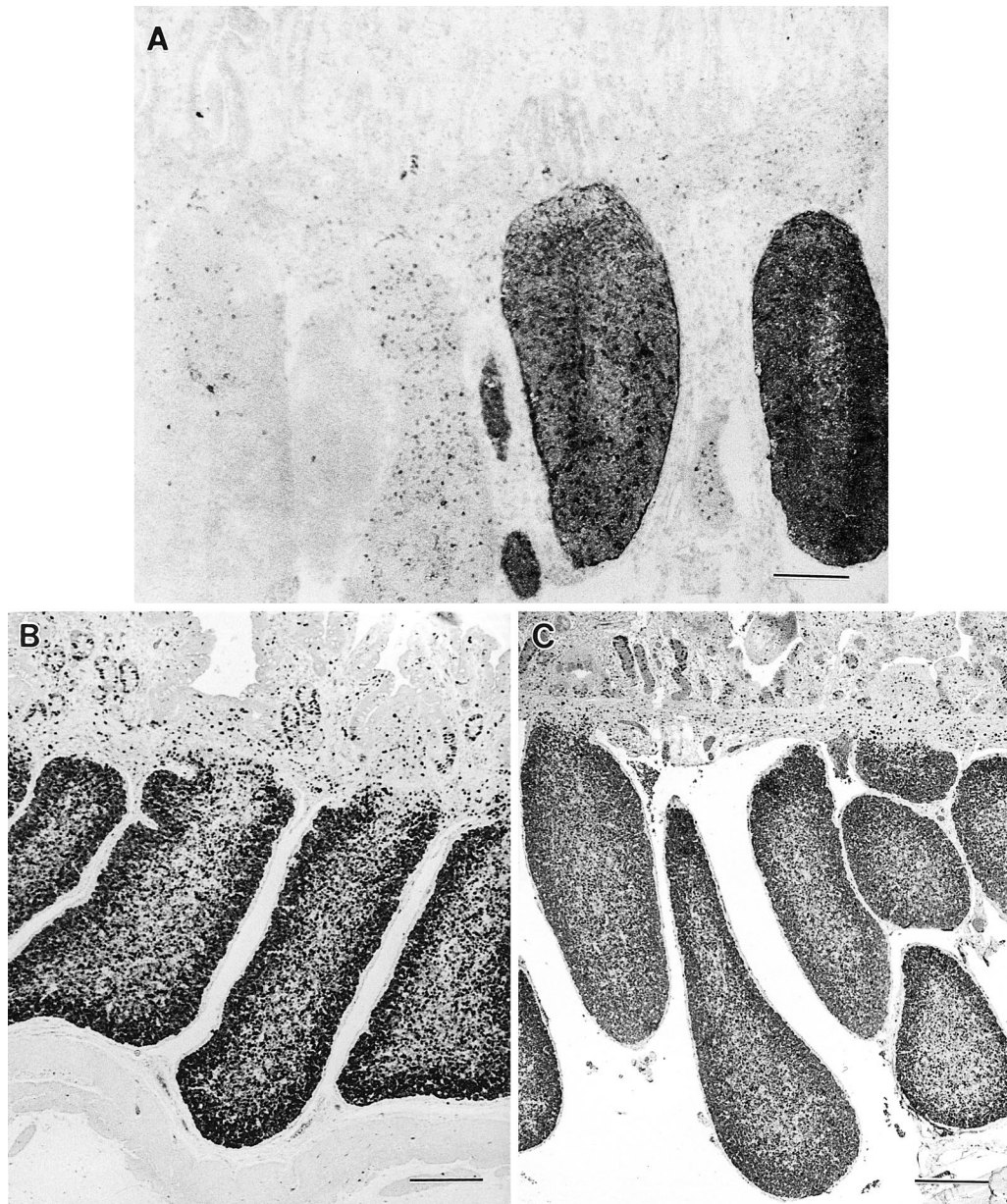

Figure 5. Distribution of BVDV antigen in the thymus at day 6 pi (calf No. 6). Viral antigen is present in lymphocytes and in cells with dendritic morphology in the thymic cortex. The number cells containing viral antigen varies between individual lobules (L1, L2, and L3). Bar = 50 μm.

as well as gut-associated lymphoid tissue at the ileocecal junction, in the proximal colon and rectum of calf No. 5, and in the ileal Peyer's patch of calf No. 6 (Fig. 6A). The BVDV antigen was present predominantly in lymphocytes. The number of BVDV-positive cells varied highly between individual lymphoid follicles of the ileal Peyer's patch (Fig. 6A). In several lymphoid follicles, viral antigen was present in the apical aspect only. All lymphoid follicles contained BVDV antigen in the remaining sites of gut-associated lymphoid nodules in calf No. 6. In these lymphoid follicles, viral antigen was detectable in all lymphocytes and follicular dendritic cells present. In both calves, BVDV antigen was seen in moderate numbers of lymphocytes and cells with dendritic morphology in the domes and in a few cells in the interfollicular areas in all sites with gut-associated lymphoid tissue. Small groups of epithelial cells on domes and in crypts of the adjacent mucosa contained viral antigen in gut-associated lymphoid tissue in the ileum and rectum of calf Nos. 5 and 6 and in the jejunum and proximal

colon of calf No. 6 (Fig. 7). In the duodenum of calf No. 6, a few lymphocytes in the lamina propria of villi and between crypts were positive for BVDV antigen and also in the midcolon, pericryptal cells, and small groups of crypt epithelial cells.

Between the 2 calves necropsied at day 9 pi (calf Nos. 7 and 8), there were marked differences in the number of organs and cells in which BVDV antigen was detectable (Table 1). In calf No. 7, follicular dendritic cells and macrophages in a few lymphoid follicles in the tonsil, retropharyngeal lymph node, mesenteric lymph node, jejunal Peyer's patch and gut-associated lymphoid tissue at the ileocecal junction, proximal colon, and rectum contained BVDV antigen. In the ileal Peyer's patch, BVDV antigen was detectable in every lymphoid follicle. Viral antigen was associated with follicular dendritic cells and apoptotic lymphocytes in the follicles (Fig. 8A, 8B). The BVDV-positive lymphocytes and cells with dendritic morphology were distributed diffusely throughout the thymus. In calf No. 8, BVDV antigen was present only
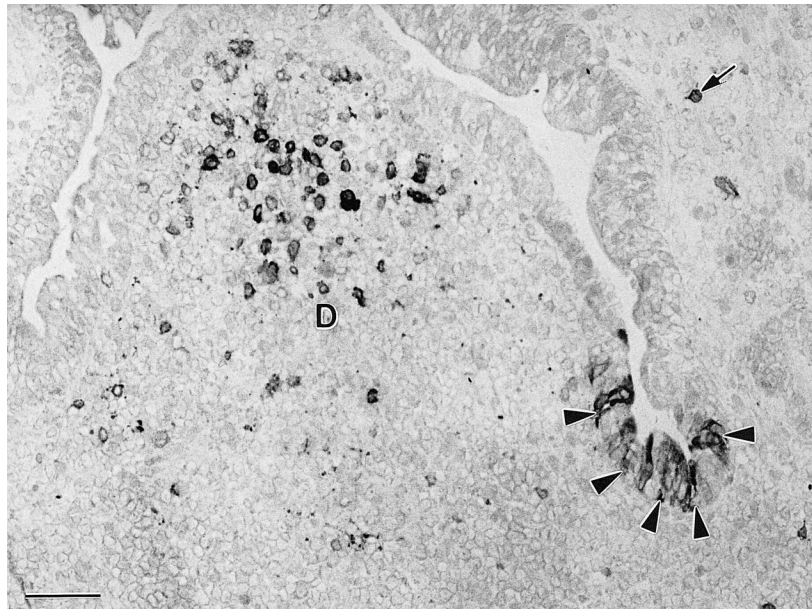
Figure 6. Distribution of BVDV antigen and cell proliferation in the ileal Peyer's patch at day 6 pi. **A**. The BVDV antigen is present in variable amounts in several lymphoid follicles. All lymphoid follicles are large and cellular, independent of the number of cells containing viral antigen. Viral antigen is also present in domes and interfollicular areas. Calf No. 6. Bar = 200 μm. **B**. Numerous proliferating cells as indicated by reactivity for Ki-67 antigen are present in the large lymphoid follicles. Calf No. 6, paraplast section, Ki-67 antigen. Bar = 200 μm. **C**. Proliferation in lymphoid follicles from the ileal Peyer's patch of a control calf for comparison. Calf No. 2, paraplast section, Ki-67 antigen. Bar = 200 μm.

in a few macrophages and follicular dendritic cells of a few lymphoid follicles in the jejunal and ileal Peyer's patches and multifocally in cells with dendritic morphology in the thymus.

At day 13 pi, BVDV antigen was detected focally in epithelial cells of a tonsillar crypt in calf No. 9. In the thymic cortex of calf No. 10, several cells with dendritic morphology were antigen positive (Table 1).

Histological findings and proliferation in lymphoid tissues. No significant histological lesions or changes

in the pattern of proliferation were seen when comparing the calves at day 3 pi to the controls. No or subtle changes were seen at day 6 pi (Fig. 6B, 6C): lymphoid tissues had large lymphoid follicles, but an increased number of macrophages containing apoptotic bodies were present in a few lymphoid follicles in the tonsils, retropharyngeal, mandibular, and mesenteric lymph nodes, and variable gut-associated lymphoid tissue sites. In the tonsil, retropharyngeal, mandibular, and mesenteric lymph nodes of calf No. 6 and

Figure 7. Distribution of BVDV antigen in the intestinal mucosa at day 6 pi (calf no. 6). Viral antigen is present in the follicle-associated epithelium (arrowheads), within the dome (D) and in the adjacent lamina propria (arrow, example) of a jejunal Peyer's patch. Bar = 50 μm.

in the mesenteric lymph node of calf No. 5, proliferation as detected by labeling for Ki-67 antigen was decreased in a few large lymphoid follicles.

At day 9 pi, marked lesions were seen in all sites with gut-associated lymphoid tissue and in the thymus of both calves. There were only a few normal lymphoid follicles in the gut-associated lymphoid tissue; most had different degrees of depletion and decreased proliferation varying from mild to severe (Fig. 9). The associated domes were also depleted of lymphocytes. A multifocal increase in numbers of apoptotic bodies and a corresponding decrease of proliferation, which was severe in calf No. 7 and mild in calf No. 8, were seen in the thymic cortex (Fig. 10). The severity of changes in the tonsils and lymph nodes was different between calf Nos. 7 and 8. In calf No. 7, several large but centrally depleted lymphoid follicles were present in the tonsil and all the lymph nodes collected; this was only seen in the mesenteric lymph node of calf No. 8. In both calves necropsied at day 6 pi (calf Nos. 7 and 8), more Ki-67–positive cells were present in the interfollicular areas of the tonsil and the paracortex of the lymph nodes than in the controls (calf Nos. 1 and 2).

At day 13, various degrees of recovery were seen. In the tonsils and lymph nodes of both calves, large lymphoid follicles and hyperplasia of the interfollicular zone and paracortex, respectively, associated with numerous Ki-67–positive cells were present. In calf No. 9, some of the large lymphoid follicles had depleted centers with decreased proliferation. The lesions

in the gut-associated lymphoid tissue varied between calf Nos. 9 and 10. In calf No. 9, most lymphoid follicles in the gut-associated lymphoid tissues were severely depleted (Fig. 11A), some contained groups of proliferating lymphoblasts. In calf No. 10, most lymphoid follicles in the gut-associated lymphoid tissues were of normal appearance (Fig. 11B). A few depleted lymphoid follicles containing groups of lymphoblasts were observed only in the jejunal Peyer's patch (Fig. 11C–11E), and in the ileal Peyer's patch, there were a few completely depleted lymphoid follicles (Fig. 11B). In the thymic cortex of calf No. 10, multiple foci with reduced numbers of Ki-67–positive cells were present. Multiple small groups of lymphocytes, plasma cells, and macrophages were seen in the interstitium of the renal cortex of calf Nos. 3, 4, 6, and 7.

Discussion

Inoculation of calves with BVDV 2 strain 28508-5 resulted in an infection without obvious clinical signs. The transient temperature elevation was detected, only because body temperature was taken daily. Thus, the infection would most likely have passed unrecognized under field conditions. Although this course of infection predominates after exposure to BVDV 1,[2,3,6,7] severe acute BVD often with hemorrhages has been reported in BVDV 2 infections.[1,5,8,9,12,16,25,28,29,31,37]

There were no macroscopic lesions in the controls and the animals necropsied at days 3 and 6 pi. The large tonsils and lymph nodes seen in this study are common findings in young animals. Therefore, the
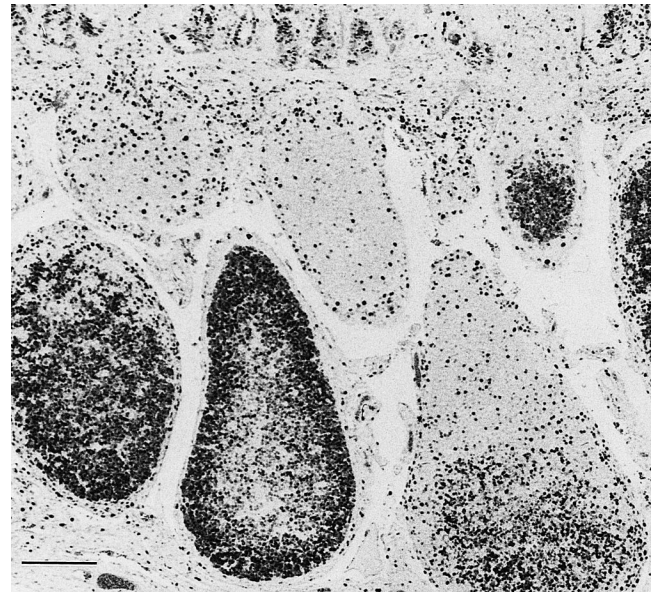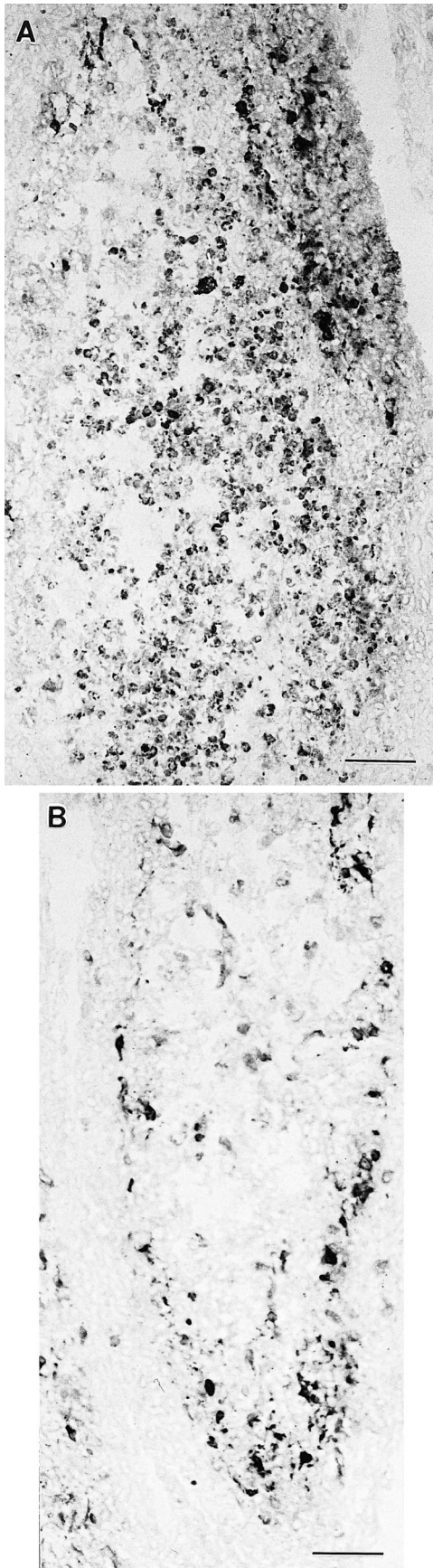
Figure 9. Tissue alterations in the ileal Peyer's patches at day 9 pi (calf No. 7). Proliferating cells are variably reduced in number in several lymphoid follicles. Paraplast section, Ki-67 antigen. Bar = 200 μm.

mild to moderate reduction in size of lymph nodes and tonsils and in thickness of Peyer's patches in the animals at days 6 and 9 pi indicating depletion of these lymphoid tissues might easily be missed.

No changes in platelet numbers were noted after inoculation with BVDV 2 strain 28508-5 consistent with the lack of hemorrhages. This is clearly different from other cases of BVDV 2 infection in which thrombocytopenia occurs regularly.[1,5,9,12,16,25,28,37] The cause of thrombocytopenia in BVDV 2 infections is still not completely resolved. Reduced production of platelets by megakaryocytes, increased consumption of platelets in the periphery and functional defects of platelets have been suggested.[9,31,39] Although in cases of BVDV 2 infections resulting in thrombocytopenia, viral antigen was detected in megakaryocytes,[25,27] no infection of bone marrow including megakaryocytes was seen after inoculation with BVDV 2 strain 28508-5. Infection of megakaryocytes may be crucial to the development of thrombocytopenia.

The lack of clinical signs and gross lesions was apparently not due to the inability of BVDV 2 strain 28508-5 to spread and replicate in host tissues. Viral antigen was detectable at day 3, as has been reported

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Figure 8. Distribution of BVDV antigen in the ileal Peyer's patch at day 9 pi (calf No. 7). **A**. Viral antigen is associated with apoptotic lymphocytes in a lymphoid follicle. Bar = 50 μm. **B**. Viral antigen is present in follicular dendritic cells and macrophages in depleted lymphoid follicles. Bar = 50 μm.
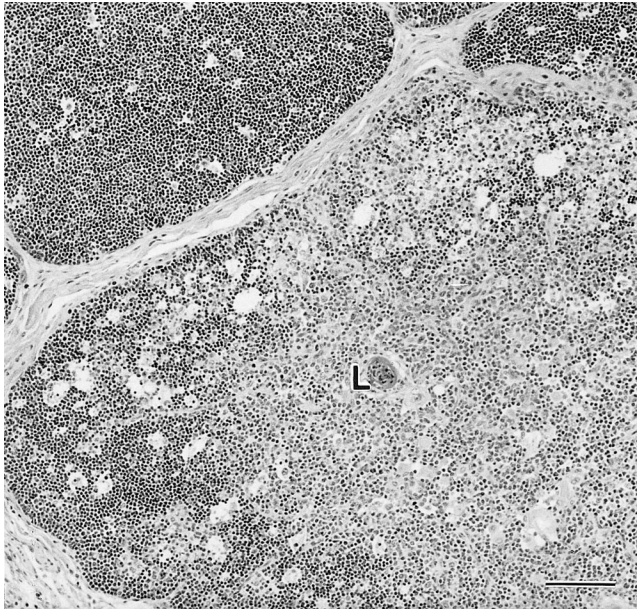
Figure 10. Tissue alterations in the thymus at day 9 pi (calf no. 7). A severely depleted thymic lobule (L) is surrounded by several normal lobules. Cell loss is predominantly seen in the cortex. Paraplast section, HE. Bar = 100 μm.

for other more virulent strains of BVDV.[25,36] In this early phase of infection, viral antigen was present in the same target cells as was observed with virulent strains of BVDV 2[25] and cp strains of BVDV in mucosal disease.[23]

The BVDV 2 strain of low virulence did not remain restricted to its entry site but became widespread in lymphoid organs throughout the body. Compared with a highly virulent strain, spread of the disease was slower and the spectrum of tissues infected more limited in the strain of low virulence.[25] In the tonsil, lymph nodes, spleen, and gut-associated lymphoid tissue, viral antigen was predominantly seen in the lymphoid follicles indicating association with B lymphocytes and follicular dendritic cells, whereas only few cells in the T cell–dependent areas contained viral antigen. The low infection rate of T lymphocytes in secondary lymphoid tissues cannot be explained by a lack of affinity of BVDV 2 strain 28508-5 for T lymphocytes in general because many T lymphocytes in the thymus were positive for viral antigen and may reflect a preference for cells with very high replication rates.

Antigen of the BVDV 2 strain used in this study was not completely restricted to lymphoid tissues but was also present multifocally in the intestinal mucosa. This indicates that BVDV 2 of low virulence is able to replicate in the digestive tract, and virulence is not dependent on the ability of BVDV to infect the digestive tract.

After day 6 pi, BVDV 2 strain 28508-5 was rapidly cleared from most tissues, thereby also preventing fur-

ther spread in the digestive tract. This behavior of the strain of low virulence is remarkably different from more virulent strains of BVDV 2 that spread to all compartments of the lymphoid tissues and most tissue types and organs of the host after an initial replication in the lymphoid follicles.[12,25,27,28,37] Because strain 28508-5 was cleared before this massive spreading occurred, it was never seen in the respiratory tract, in endocrine organs, in the skin, and, as mentioned above, in the bone marrow. Matching this observation, there were no signs of pneumonia in any of the inoculated calves. The lack of viral presence in the skin suggests that skin biopsies will not detect acute infections with BVDV strains of low virulence.[34]

The clearance of virus coincided with destruction of infected lymphocytes and the resulting depletion of lymphoid follicles and thymus. The cause of death of infected lymphocytes in the lymphoid follicles is still unresolved because the cells were infected with ncp BVDV. Furthermore, lesions had a delayed onset: viral antigen was present for a limited time in almost all cells of some lymphoid follicles without inducing morphological changes. Because lesions developed in lymphoid follicles and thymus, sites where cells are programmed to undergo apoptosis if they are not rescued,[26] subtle changes in cellular metabolism induced by replication of ncp BVDV of low virulence might accumulate and eventually result in apoptosis. On the other hand, the delayed onset might also be indicative of a host response causing the lesions. Antigen-specific CD8^{+} and CD4^{+} cytotoxic T lymphocytes may induce apoptosis of infected cells.[11,15] CD4^{+} T lymphocytes appear to be more important for the control of BVDV infection because only depletion of CD4^{+} T lymphocytes caused prolonged viremia.[20]

After the loss of infected lymphocytes, antigen was detected in follicular dendritic cells and macrophages in the lymphoid follicles and in dendritic cells in the thymic cortex. The resistance of these cells to destruction is most likely related to their lower replication rate or lack of replication.[21] Ultrastructural observations in mucosal disease suggest that a very rapid loss of B lymphocytes in lymphoid follicles sometimes induces destruction of follicular dendritic cells causing cystic changes of lymphoid follicles.[38] The depletion of lymphoid follicles after infection with BVDV 2 strain 28508-5 appeared to proceed slowly enough for retention of follicular dendritic cells. The presence of follicular dendritic cells allowed a repopulation with lymphocytes as has been shown after depletion of Peyer's patch follicles by dexamethasone.[14] The recovery progressed with different speed in the individual animals and was not completed at the end of the experiment at day 13 pi. A prolonged presence of viral antigen was seen in the tonsillar epithelium and in dendritic
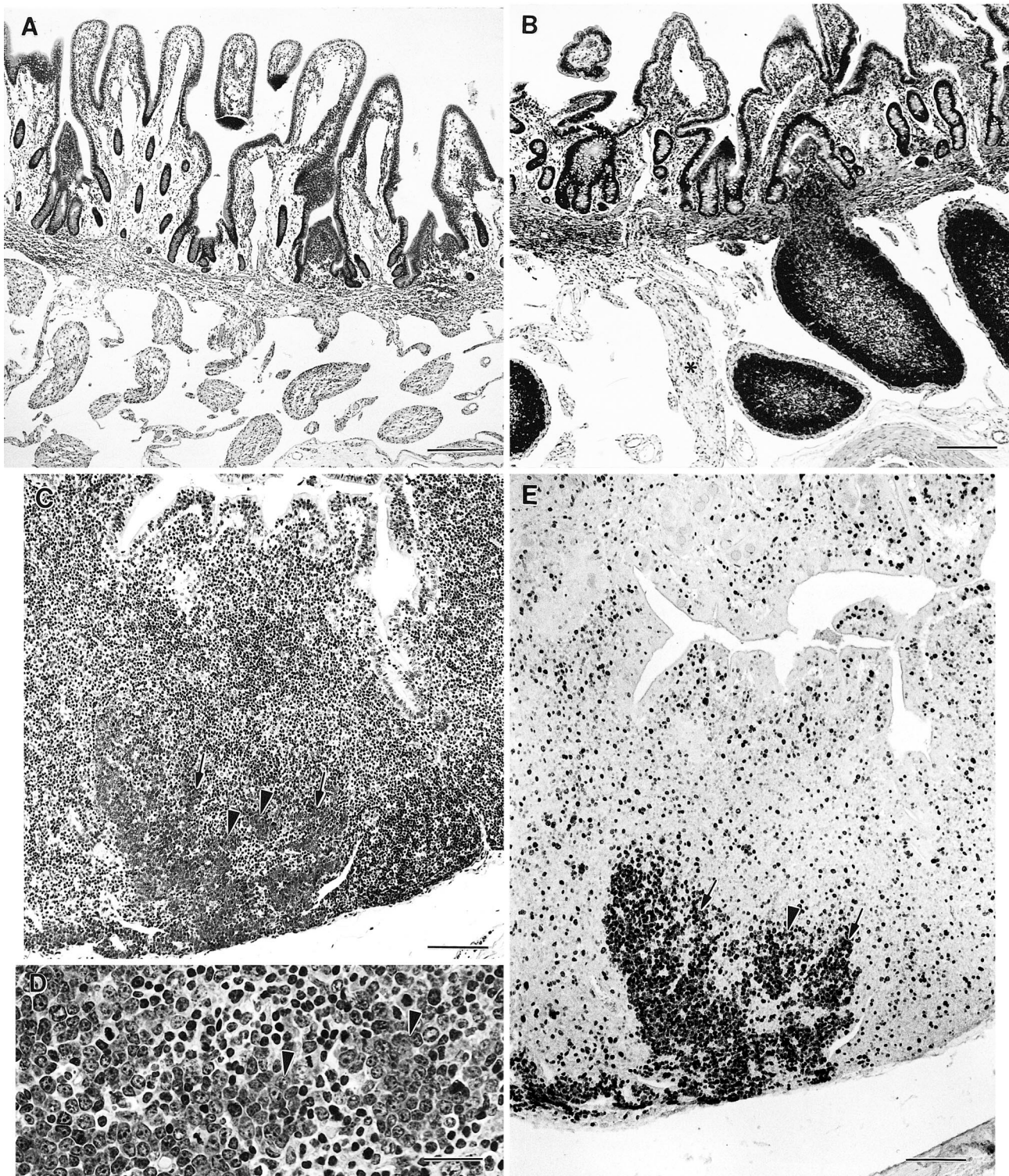
Figure 11. Tissue alterations in Peyer's patches at day 13 pi. **A**. Severe depletion of lymphoid follicles in the ileal Peyer's patch of calf No. 9. Paraplast section, HE. Bar = 200 μm. **B**. Most lymphoid follicles are large and cellular in the ileal Peyer's patch of calf No. 10; there is a single severely depleted lymphoid follicle (*). Paraplast section, HE. Bar = 200 μm. **C**. Multiple aggregates of lymphoblasts (arrows and arrowheads) in a lymphoid follicle of a jejunal Peyer's patch of calf No. 10 indicate repopulation. Paraplast section, HE. Bar = 100 μm. **D**. Higher magnification of lymphoblast aggregates indicated by arrowheads in B. Paraplast section, HE. Bar = 25 μm. **E**. Lymphoblast aggregates (arrows and arrowhead) in a consecutive section of the lymphoid follicle from B are positive for Ki-67 antigen. Paraplast section, Ki-67 antigen. Bar = 100 μm.

cells in the thymus. Although BVDV antigen was present in tonsil and thymus, virus was not detectable in the buffy coat at day 13 pi.

In conclusion, there are marked differences in spreading and clearance between BVDV 2 strains of different virulence. Despite the lack of clinical signs indicating an infection, BVDV 2 strain 28508-5 transiently compromises the immune system and thus possibly predisposes cattle to secondary infections.

Sources and manufacturers

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